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COMPOSITION STUDIES ON TOBACCO.
XX,*—Bases of Cigarette Smoke

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The major components in the basic fraction of cigarette smoke studied herein were pyridine, isomeric picolines, 3-vinylpyridine, nicotine, nornicotine, myosmine and 2,3′-bipyridyl. Evidence for the presence of several isomeric lutidines, pyrrole, nicotinonitrile, 3-ethylpyridine, methyl 3-pyridyl ketone, metanicotine and anabasine was also obtained. The levels of the major bases in smoke from blended or unblended flue-cured, burley, Maryland and Turkish cigarettes showed some differences which were discussed. The amounts of certain bases in the smoke from flue-cured cigarettes having different organoleptic properties were determined and possible relationships between smoke flavour and chemical composition were discussed.

Introduction

Recently published studies in this series have described the composition of the total volatile neutral[1–3] and acidic[4–7] fractions of cigarette tobaccos and smoke condensates. In a continuation of this work, the bases of cigarette smoke are now reported.

The complexity of the nitrogen-containing fraction of tobacco smoke is well known and many compounds have been identified therein,[8, 9] including ammonia, aliphatic amines, pyridine, alkylpyridines, acylpyridines, nicotine and related alkaloids, quinoline, indoles, carbazoles, harmanes, acridines and others. With a few exceptions, composition studies on the bases have been limited to the isolation of a single compound or small groups of compounds rather than a survey of the entire basic fraction such as can be accomplished by gas chromatography. Glock & Wright[10] and Kobayashi *et al.*[11] may have performed such a survey but their works have not been detailed. Quin[12] has surveyed mainly the alkaloidal substances in burley tobacco smoke. Therefore, a method was developed at this laboratory for studying the entire basic fraction by gas chromatography,[13] and was initially applied to cigar smoke. As a sequel, the bases of cigarette smoke from tobaccos of different types and of different organoleptic properties have now been investigated.

* Part XIX. *Tob. Sci.*, 1964, **8**, 82

Experimental

Cigarettes and fractionating procedure

The tobaccos, their physical characteristics, the smoking conditions, and the collection of the smoke condensate have been described previously.[2, 7] For the tobacco types, 105 cigarettes were smoked. For the tobacco grades, 45 cigarettes of all samples were smoked except for Aromatic A of which 26 were smoked. Values are expressed as the amount in the smoke condensate obtained from smoking 55% of the cigarette weight. Blend FBM consisted of 60% flue-cured, 35% burley and 5% Maryland tobaccos. Blend FBMT contained 40% flue-cured, 35% burley, 5% Maryland and 20% Turkish tobaccos.

The smoke condensate from each set of cigarettes was collected in cooled traps and removed therefrom by successive washings of the traps with ether (total vol., 150 ml.) and 1N-hydrochloric acid (total vol., 150 ml.). The ether washings were combined and extracted five times with 1N-hydrochloric acid (total vol., 250 ml.) and all hydrochloric acid extracts were combined.

The pooled acidic solution of bases (total vol., 400 ml.) was extracted continuously with ether (500 ml.) for 20 h. to remove neutral substances, after which the pH of the acidic solution was adjusted to 12·0 by the addition of sodium hydroxide. The liberated bases were then removed from this alkaline solution by continuous extraction with ether (500 ml.) for 20 h. The ether solution of bases was dried over anhydrous magnesium sulphate, and concentrated to 5 ml. on the Stedman and spinning band columns previously described.[1] Aliquots of the concentrate were then studied by gas chromatography. The size of the aliquots ranged from 1 to 40 μl. depending on the number of smoked cigarettes represented by the sample and on the type of gas chromatographic detection system used.

Gas chromatography

Several chromatographic instruments and conditions were used in identifying and estimating the amounts of the bases in smoke. Table I is a summary of pertinent data. The optimum conditions are indicated in each instance. Instrument C had two columns each having a thermal conductivity and a flame ionisation detector and was operated in conjunction with a dual

Table I

Chromatographic conditions used in separation of bases in cigarette smoke

Conditions*	Instrument* A	B	C
Principal use			
Qualitative, low-boilers	X	X	X
Qualitative, high-boilers	X	X	
Quantitative, low-boilers			X
Quantitative, high-boilers	X†	X	
Columns, number	Single	Dual	Dual
Columns, size	¼ in. × 8 ft.	⅛ in. × 6 ft.	⅛ in. × 8 ft.
Column substrates and temperatures			
Carbowax 20M (CARB)	20 min. at 100° 5°/min. to 235°	—	45 min. at 113° 6°/min. to 240°
Silicone grease (SG)	Same as CARB		—
Polypropylene glycol (PPG)			
Low-boilers	—	100°	—
High-boilers	—	190°	—
Injector temperature	260°	160° (L-B) 205° (H-B)	260°
Detector, temperature	260°	‡	260°
Detector, type	TC	FI	TC + FI
Detector, no. per column	1	1	1-TC + 1-FI
Bridge current (mA)	150	—	100
Carrier gas (He) flow (ml./min.)	60 (CARB) 75 (SG)	60 (L-B) 45 (H-B)	60
Pressure, air (p.s.i.)	—	40	12
Pressure, hydrogen (p.s.i.)	—	20	7 (sens.), 5 (ref.)

* TC = thermal conductivity, FI = flame ionisation, sens. = sensing column, ref. = reference column. 'Low-boiling' components (L-B) refer to peaks eluting before nicotine, and 'high-boiling' components (H-B) refer to all peaks eluting after low-boiling components.

† Nicotine was the only alkaloid determined with these conditions.

‡ Detector and columns are in the same oven.

channel recorder ; this instrument gave the greatest sensitivity and least base-line drift and was employed for estimations of low-boiling components. Quantitative data were obtained by measuring peak heights of unknowns and obtaining values from concentration–peak height curves of known compounds.

Paper and thin-layer chromatography

Paper chromatographic methods were those of Tso & Jeffrey,[14] and of Brandsch ;[15] in our hands, the latter method was preferable for the lower-boiling pyridine derivatives. Thin-layer chromatographic separations of the basic fraction of cigarette smoke were performed on 8 in. × 8 in. glass plates coated with silica gel G which were developed in either of two solvents : 9 : 1 (v/v) chloroform–95% ethanol[16] or 1 : 1 (v/v) 95% ethanol–0·2M-acetate buffer (pH 5·6).[17]

Individual gas chromatography peaks and groups of peaks from the total basic fraction were collected in U-tubes cooled in dry ice–acetone and the collected materials were chromatographed by the thin-layer and paper-chromatographic methods. With both methods, spots were made visible by spraying the chromatograms with *p*-aminobenzoic acid, followed by exposure to cyanogen bromide vapours.[14] Attempts to chromatograph the total basic fraction were unsuccessful since gross streaking rather than discrete separation was observed.

Spectral studies

Known and unknown compounds were generally dissolved in 0·1N-hydrochloric acid for ultra-violet spectral measurements and were spread as a film on sodium chloride disks for infra-red spectral determinations.

Results and discussion

Identities of major components

A chromatogram of the basic fraction of smoke from commercial American cigarettes is shown in Fig. 1. This separation was made on the instrument having a single column and thermal conductivity detection and is presented to illustrate the complexity of the entire fraction. A peak possibly identical with 2,3′-bipyridyl is present in the eluate after peak 34 and is not seen in Fig. 1. Fig. 2 is a portion of a chromatogram of the basic fraction of smoke from cigarettes made from Turkish tobacco obtained using an instrument with dual columns and simultaneous flame ionisation and thermal conductivity detection. This chromatogram compared with Fig. 1 shows the improved sensitivity and eluting pattern (less baseline drift, better resolution of some components, etc.) for the lower boiling constituents and is presented to illustrate conditions under which quantitative measurements were made.

Evidence for the identities of certain peaks, based on the following criteria, is presented in Table II : vapour-phase co-chromatography (3 substrates) with known compounds ; paper- and

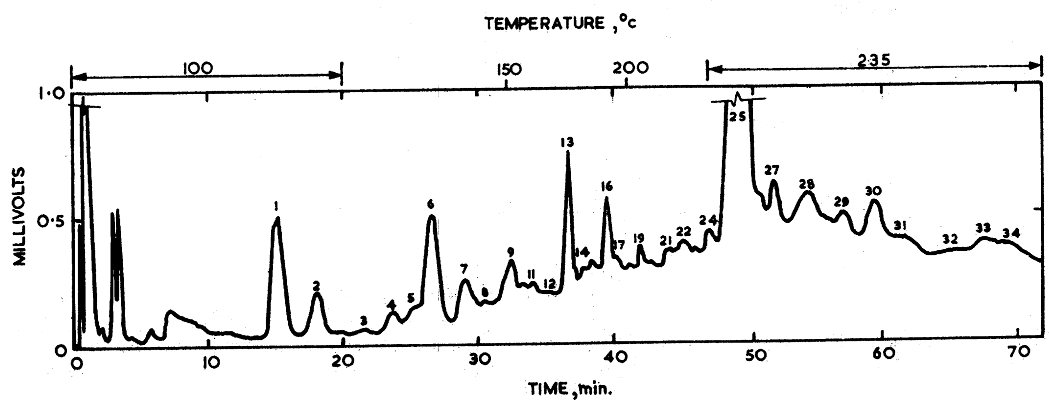

Fig. 1.—*Chromatogram of bases from smoke of commercial brand of American cigarettes*

Aliquot equivalent to 7·35 cigarettes was injected. Operating conditions and peak identities are described in Tables I and II

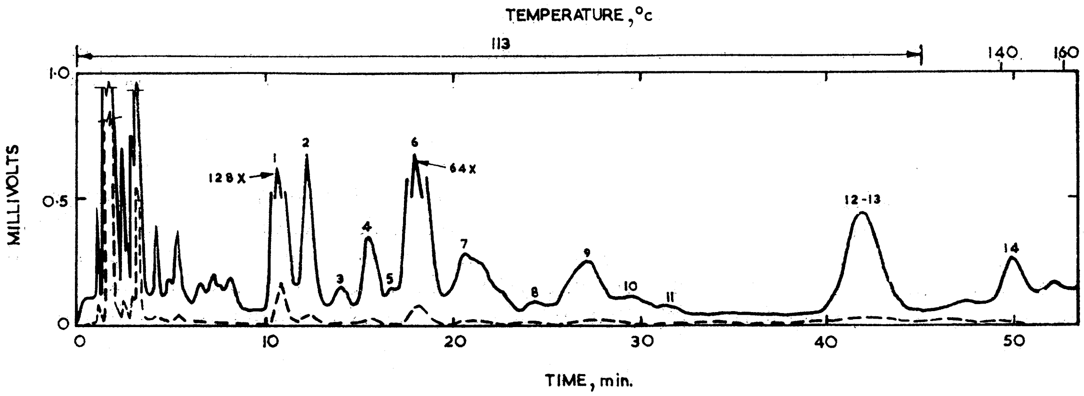

FIG. 2.—*Chromatogram of certain volatile bases (see text for definition) from smoke of cigarettes containing all Turkish tobacco*

Aliquot equivalent to 0·012 cigarettes was injected. Operating conditions and peak identities are described in Tables I and II

Flame ionisation ——— (32×)
Thermal conductivity - - - - - (2×)

thin-layer-chromatographic comparisons of R_F and colour reactions with authentic components; and ultra-violet spectral comparisons with known substances. Additional indirect evidence was obtained from infra-red spectral comparisons of identical peaks recently[13] isolated and identified

Table II

Identification of cigarette smoke bases

Peak no.	Identity	Chromatography*					Spectra*	
		Vapour phase			Paper	Thin-layer	UV	IR
		CARB	SG	PPG				
1	Pyridine	+	+	+	+	+	+	+
2	α-Picoline	+	+	+	+	+	+	+
3	2,6-Lutidine	+	+	+	+	+	+	+
6	β-Picoline γ-Picoline	{+ +}		{+ +}	{+ +}	{+ +}	{+ +}	{+ +}
7	2,4-Lutidine 2,5-Lutidine	{+ +}	 +	 +				{+ +}
8	2,3-Lutidine 2,4,6-Collidine	{+ +}	+ 	{+ +}				+
9	3-Ethylpyridine	+				+		+
11	3,5-Lutidine	+	+	+				
12	3,4-Lutidine	+	+	+				
13	3-Vinylpyridine	+		+	+	+	+	+
14	Pyrrole	+		+				
21	Pyridine-3-aldehyde			+				
22	Nicotinonitrile	+		+				
24	Methyl 3-pyridyl ketone	+		+				
25	Nicotine	+	+	+	+	+	+	+
27	Nornicotine Myosmine	{+ +}	{+ +}	{+ +}	+ +	 +	{+ +}	{+ +}
30	Anabasine	+	+	+			+	
31	Metanicotine	+		+				
—	2,3-Bipyridyl	+		+			+	+

* Comparisons of known compounds and unknown peaks by gas co-chromatography on 3 columns (CARB = Carbowax, SG = silicone grease, PPG = polypropylene glycol), by paper (2 solvent systems for pyridine and 1 for all others) and thin-layer chromatography, by ultra-violet (UV) spectra and by extrapolation of infra-red (IR) spectra of identical peaks in cigar smoke (see text).

+ = Known and unknown compounds showed identical characteristics. Bracketed combinations indicate either failures in resolution or spectral characteristics suggestive of a mixture of the indicated compounds.

in cigar smoke condensate, which is, in general, qualitatively similar to cigarette smoke with respect to major components. A large number of peaks were unidentified, including several which were eluted before pyridine (Fig. 2). Piperidine and pyrrolidine eluted in this region, but the presence of these bases in the smoke could not be verified. Probably many of the peaks contain other components in addition to those known or suspected therein, e.g., a probable third component in peak 7 (Fig. 2).

Difficulties in gas-chromatographic resolution were encountered with certain combinations of bases as shown in Table II. Myosmine and nornicotine separated slightly when neither was in great excess; however, good separation was obtained on paper chromatograms under all conditions. Resolution of the bases of tobacco and its smoke by thin-layer chromatography has not been previously reported. The method appears to be of value but no extensive study of its potentialities has been made.

Oxynicotine could not be determined by gas chromatography possibly because of thermal degradation,[12] and nicotinic acid and nicotinamide were not recovered by the method due possibly to unfavourable partition coefficients. Indoles,[18] carbazoles,[18, 19] acridines,[19] pyrocoll,[18, 20] harman[21] and norharman[21] might have been present among the higher-boiling unidentified peaks, although some of these compounds might not have been eluted under the chromatographic conditions or been removed by aqueous acid.

Of the compounds listed in Table II, nicotinonitrile and metanicotine have not been reported previously in cigarette smoke.[8, 9] Although the presence of 3-vinylpyridine in cigarette smoke has been cited by others,[10, 11] the present report presents the first details of its isolation. The occurrence of 3-vinylpyridine, nicotinonitrile and metanicotine among the pyrolytic products of nicotine,[22–24] nornicotine[25] and myosmine[25] is well known.

Amounts in smoke from types and grades

Most American cigarettes and many cigarettes produced in other countries contain blends of various tobacco types. The principal types in the former case are flue-cured (bright), burley, Maryland and Turkish, each of which is believed to contribute specific properties influencing the burn rate, flavour, aroma, etc. of the blended cigarette. Also, samples of a type are usually graded for their relative suitability for smoking purposes, and there is considerable interest in determining the nature of the chemical constituents which contribute to the observed differences in types and grades.

Types.—Table III shows the amounts of the major constituents in smoke from blended and unblended cigarettes of the above types. The values are uncorrected for losses in the isolation procedure which vary from 0 to 30% for components eluted in the range from pyridine to nicotine. The losses have been shown to occur mainly during evaporation of the solvent (ether). The compounds in Table III are arbitrarily divided into 'volatile bases', which are the simple pyridine derivatives, and 'alkaloids', which are the structures more closely related to nicotine. The terms, 'volatile bases' and 'total volatile bases', as used here, should not be confused with identical terms employed in quality control work to describe titratable bases which are released on steam distillation of strongly alkalised tobacco or smoke.[26]

Values for 3-ethylpyridine and 3-vinylpyridine were determined with 2,3-lutidine and 3,4-lutidine, respectively, as standards since these combinations have similar chromatographic characteristics. Totals were calculated by using maximum values in cases in which no exact value could be assigned, e.g., 1·5 μg. was employed for 2,6-lutidine (burley, Maryland, etc.) and 3-ethylpyridine (flue-cured). Although a statistical study of variability was not conducted with this method, it appears to be less reproducible than the recently published method for volatile acids,[6] but is suitable for detecting large differences and trends.

The major components among the volatile bases of the smoke condensates were pyridine, isomeric picolines and 3-vinylpyridine. Among the unblended cigarettes, burley and Turkish contained more of these compounds than flue-cured and Maryland, but the differences were not great. In contrast to the blend without Turkish, the blend containing Turkish had much more total volatile bases than any of the constituent tobaccos, indicating that Turkish tobacco enhances the evolution of these compounds in such blends during burning. A similar pattern of enhancement was observed previously with certain volatile (C_4–C_6) acids in this blend.[6]

Table III

Levels of certain bases in smoke condensates from tobaccos of various types

Components	Micrograms per cigarette					
	Flue-cured	Burley	Maryland	Turkish	Blend* FBM	Blend* FBMT
Volatile bases						
Pyridine	40	35	27	35	35	52
α-Picoline	11	8·5	6·7	16	9	31
2,6-Lutidine	<1·5	<1·5	<1·5	1·7	<1·5	<1·5
β- and/or γ-Picoline	24	46	37	51	34	51
2,4- and/or 2,5-Lutidine	3·4	5·1	5·9	15	9·0	14
2,3-Lutidine and/or 2,4,6-collidine	<1·5	<1·5	<1·5	1·7	<1·5	<1·5
3-Ethylpyridine	<1·5	3·6	3·6	2·5	2·1	1·9
3-Vinylpyridine	13	23	17·0	15	18·0	28
Total†	95·9	124	100	138	110	181
Alkaloids						
Nicotine	3500	4500	2900	3300	3100	3300
Nornicotine–myosmine	11	50	38	15	18	24
Anabasine	5·7	11	9·5	6·2	3·3	9·5
2,3′-Bipyridyl	11·0	27	11	7·2	22	22
Total (without nicotine)	27·7	88·0	58·5	28·4	43·3	55·5

* Blends of flue-cured, burley and Maryland with (FBMT) or without (FBM) Turkish tobaccos. See Experimental section for exact composition.

† See text for method of calculating totals; individual and total values were rounded off to two and three digits, respectively.

In the alkaloidal fraction, nicotine was the major component, as expected. The relative nicotine levels for the smoke condensates from the four unblended tobaccos deviated slightly from the patterns reported by others[26, 27] in that in Turkish smoke the level was somewhat high. However, such comparisons may be questionable since the composition of tobacco types, including Turkish,[28] varies markedly.* Levels of total alkaloids (without nicotine) were higher for unblended burley and Maryland tobacco smokes. Between the blended cigarettes, there appeared to be no marked enhancement of minor alkaloid formation in the blend with Turkish tobacco, although this blend contained a higher level than the other blend.

Grades.—Table IV shows the levels of the major bases in smoke from grades of all-flue-cured cigarettes which are popular in Great Britain and certain Commonwealth nations. The cigarettes, which differed in relative smoke flavour (*vide infra*), were made from unblended tobaccos having different degrees of leaf aroma. The smoke condensates from these cigarettes were relatively low in anabasine and certain other bases listed in Table II, and accurate values for these components could not be obtained by reason of the limited supply of cigarettes.

The values for the total volatile bases generally paralleled the corresponding nicotine levels although exceptions were noted. All cigarettes made from 'Aromatic' bright tobacco had higher levels of the volatile bases and nicotine than those from 'Aroma-deficient' samples, although the difference between Aromatic B and Aroma-deficient A was relatively small and possibly insignificant.

The relative flavour ratings for the cigarettes and the relative ratings of total volatile bases, total nicotine and other components are shown in Table V. In general, there was some relation between smoke flavour and the amounts of volatile bases and nicotine. However, in the case of the volatile bases, the differences among some samples were relatively small (Table IV) and were probably of questionable significance.

Table V also gives previously published ratings for total volatile acids: the latter components are a series of volatile fatty acids between acetic and n-caprylic which elute progressively from diethylene glycol adipate–phosphoric acid columns and have been shown[7] to be related to leaf aroma. When these results were reported previously, it was noted that the discrepancy in the smoke flavour–smoke acid composition relationship shown by Aroma-deficient B cigarettes might have been due to a deficiency of smoke bases since an acid–base balance is believed[26]

* The Turkish tobacco in the cigarettes studied herein contained 1·97% of nicotine by the gas-chromatographic method of Quin & Pappas.[32]

Table IV

Levels of certain bases in cigarette smoke condensates having different organoleptic properties

Components	μg. per cigarette*				
	Aromatic			Aroma-deficient	
	A	B	C	A	B
Volatile bases					
Pyridine	24	20	18·0	23	13
α-Picoline	4·4	3·8	7·8	1·9	4·4
β-, γ-Picoline	33	30	29	29	12
3-Vinylpyridine	12	11	17	6·9	5·6
Total	73·4	64·8	71·8	60·8	35·0
Alkaloids					
Nicotine	5400	4400	4200	3100	1500
Nornicotine–myosmine	15	15	11	17	13
2,3′-Bipyridyl	15	13	8·8	15	<8

* Cigarettes made from all-flue-cured tobaccos graded as 'aromatic' or 'aroma-deficient', which yielded smoke of varying flavour (see Table V).

Table V

Relationships between relative smoke flavour and relative amounts of certain bases or acids in flue-cured cigarettes

Cigarettes	Relative rating*			
	Smoke flavour	Total volatile bases	Total nicotine	Total volatile acids
Aromatic A	1–2	1	1	1
Aromatic B	3	3	2	4
Aromatic C	1–2	2	3	3
Aroma-deficient A	4	4	4	5
Aroma-deficient B	5	5	5	2

* 1 = highest flavour or level of indicated components.
1–2 = indistinguishable in flavour. See text for definitions of chemical groups.

necessary for a proper smoke flavour. The results in Tables IV and V tend to confirm this explanation.

Comparison of the values for cigarette smoke bases with the amounts recently obtained[13] in cigar smoke shows that, on the basis of μg./g. of tobacco smoked, cigarettes and cigars are quite similar. These findings were somewhat unexpected since smoke bases are believed to influence the 'strength' of smoke flavour,[29] and yet cigar smoke is obviously 'stronger' in taste than cigarette smoke. Apparently other basic compounds not examined above are influencing the taste. Possibly, the ammonia content of smoke plays a critical rôle in smoke strength. The ammonia level of cigar (filler) tobacco leaf may be up to 50 times greater than that of flue-cured tobacco.[27] Similarly, it appears[30, 31] that the ammonia content of cigar smoke may be higher than in cigarette smoke although comparisons of published data are of questionable validity. On the other hand, the organoleptic differences may be merely a reflection of free nicotine in the vapour phase of alkaline (cigar) and acid (cigarette) smokes.[33, 34]

Acknowledgments

The authors acknowledge the advice or assistance of Dr. S. Osman, Mr. R. L. Chrzanowski, Miss M. Mamchyn and Mr. H. C. Higman of this Division, and the co-operation of Mr. J. M. Moseley, American Tobacco Company, in providing the panel test results and cigarettes.

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